THE RELATIONSHIP OF HOST AND VIRUS IN MOLLUSCUM CONTAGIOSUM*

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Although the disease molluscum contagiosum was first described by Bateman in the second edition of his synopsis (1814) (1), and its vital nature was definitely demonstrated as long as 44 years ago by Juliusberg (2), it is only recently that the utilization of new physical and chemical tools have allowed us to understand better the true nature of the lesion.

One of the fundamental and most debated subjects in medical science concerns the nature of virus multiplication. This puzzling problem is important not only from a theoretical point of view but also because any clear light thrown upon it will undoubtedly assist us in our further investigation into the presently barren field of viral chemotherapy. For an investigation of the relationship of virus to host cell and the methods utilized by the virus in its multiplication as demonstrated by morphological and microchemical means, the disease molluscum contagiosum lends itself with particular fitness. Thus it is a superficial disease of relatively benign nature and slow progression produced by a virus belonging to the pox group; biopsy material can be obtained almost at will and with little disturbance to the patient; from the very nature of the lesion, the material so obtained consists of the lesion itself and very little else; and the lesions are very plentifully endowed with virus.

Materials and Methods

Two particular tools have been used in the study of virus multiplication in molluscum contagiosum. The first is the microchemical technics available in cytochemistry, i.e., the utilization of different staining reactions which color specifically different chemical entities and allow the structures containing such entities to be recognized under the light microscope.

For these studies the white comedo-like core, the molluscum body, which can be expressed from the center of the lesions, was used. Such cores for the cytochemical studies were fixed in absolute alcohol and imbedded in paraffin.

Most of the cytochemical studies were performed with the pyronine methyl-green stain† and the Feulgen reaction. The staining solutions were prepared as follows:

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Pyronine methyl green technic:—

Methyl green	0.15 g
Pyronine	0.25 g
Ethyl Alcohol 95%	2.5 ml
Glycerin	20.0 ml
Carbolic acid water (0.5%)	77.5 ml

To stain the material the above solution was applied for 20 minutes and the section then rinsed well in water. After differentiation and dehydration in absolute alcohol it was cleared in xylol and mounted in clarite.

Feulgen technic:—

1. The deparaffinized sections were transferred from distilled water to 1N hydrochloric acid for 5 minutes at room temperature.
2. Then into 1N hydrochloric acid, pre-warmed to 60° C for 5, 10 or 15 minutes. (The time for optimal hydrolysis varies with different tissues and different fixatives.)
3. Rinsed in fresh 1N hydrochloric acid.
4. Washed well in distilled water.
5. Placed into Schiff's reagent overnight.

(Pour 200 cc. of boiling distilled water over 1 gm. of basic fuchsin. (That sold by Coleman and Bell is satisfactory.) Cool to 50° C and filter. Add 20 cc. of 1N hydrochloric acid. Cool to room temperature. Add 2 gm. of anhydrous potassium metabisulfite. Let the stain stand overnight in the dark to decolorize. If it is not colorless to pale straw color, add $\frac{1}{4}$ to $\frac{1}{2}$ gm. of powdered charcoal, shake well and filter immediately. Keep in a brown bottle in the refrigerator. The pH should not fall below 1.8. Discard if the solution becomes pink.)

6. Transferred directly to 2 changes (5 minutes each) in the following solution:

1N hydrochloric acid	5 cc.
10% potassium metabisulfite	5 cc.
Distilled water	100 cc.

7. Washed well under tap (about 10 minutes).
8. Dehydrated, cleared, and mounted.

The second tool to be used is the electron microscope. The R.C.A. model EMU has been employed. In some cases material prepared on collodion screens has been visualized directly at magnifications from × 5,900 to × 16,700. To prepare the material the molluscum bodies are ground in an agate mortar to pulverize them. After adding 0.1 ml of saline they are ground again to make a fine emulsion. This is suspended in an additional 0.8 ml of saline and centrifuged at 2,000 r.p.m. for 20 minutes (A). The supernate from A is centrifuged at 15,000 r.p.m. for one hour (B). The supernate of B is discarded. Sediment B is re-suspended in 1.0 ml of water. The sediments of A and of B are placed on collodion membranes, allowed to settle a few minutes, and the excess fluid drained off. When dry the screens are washed once with distilled water.

In other cases the material on the collodion screen has been shadowed (i.e. coated) with a very fine layer of gold, chromium or uranium 238 according to the technic of Williams and Wyckoff (3). This gives an apparent three dimensional view of the material and allows one to appreciate the inter-relationship of some structures better than with the uncoated material.

RESULTS

In Fig. 1 is shown a typical molluscum body. As can be seen, this lies entirely on the surface of the skin. The underlying squamous epithelium is markedly hypertrophied but is not otherwise abnormal. The molluscum body is for the most part well differentiated from the epithelium of the skin. At the surface of

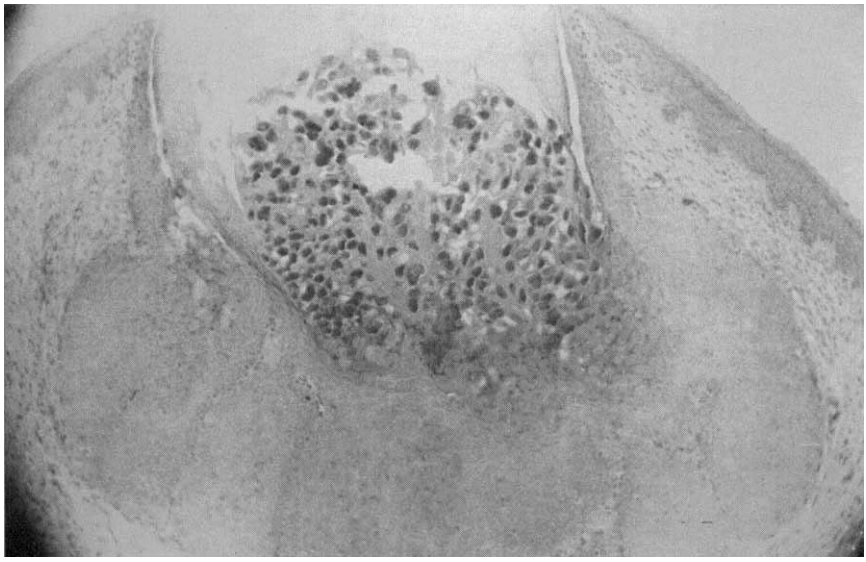

FIG. 1. BIOPSY OF MOLLUSCUM CONTAGIOSUM LESION HEMOTOXYLIN AND EOSIN × 56.5

The "core-like" central mass consists of an aggregation of inclusion bodies and amorphous debris.

the molluscum body the dark stained older inclusions are readily apparent for the most part filling completely the epithelial cells in which they lie. Broad masses of amorphous debris lie between the infected cells. The cells at the base of the body also contain inclusions which, however, are smaller and less intensely stained.

These molluscum inclusion bodies appear first as minute ovoid structures in the cytoplasm of the basal cells. The cells become distorted to produce the condition known as dyskeratosis. As the infected cells move to the surface the inclusion body enlarges until it exceeds the original size of the epithelial cell. It may reach a length of 37 micra (4). As the inclusion body grows the nucleus is displaced and compressed until it remains only as a thin crescent at the surface of the cell.

Earlier studies of these inclusions have revealed something about their structure. Thus they are described as consisting of large numbers of small bodies (5, 6, 7, 8), the molluscum elementary bodies within a gelatinous matrix (6, 7), the whole being surrounded by a carbohydrate-containing trypsin resistant membrane with a weak spot at one pole (7). Vacuoles (9) and trabeculae (6, 9) have been described in the developing inclusion body. It has more recently become clear, however, that these changes represent only the last stages of the invasion of the epithelial cell. The earlier changes have been described by Heyden and more recently have been a subject for our own investigation. Heyden made use of Feulgen stains and microspectrographic methods (10). He was able to demon-

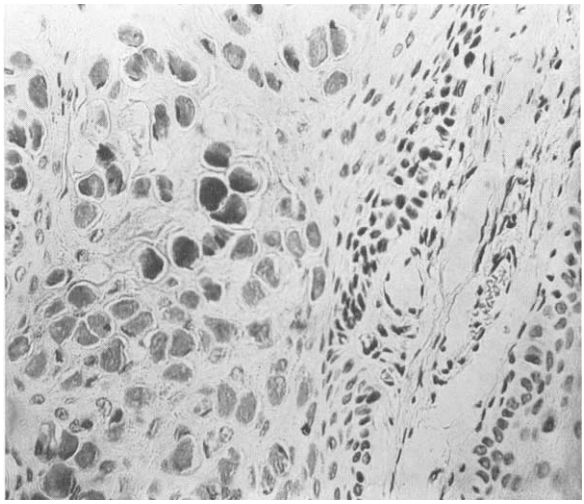

FIG. 2. BIOPSY OF MOLLUSCUM STAINED WITH FEULGEN TECHNIC X 166.7

The normal basal cells contain DNA only in their nuclei. The developing inclusion bodies contain an increasing amount of DNA in the cytoplasm which displaces all other cell structures.

strate a great increase in desoxyribonucleic acid in the inclusion body as it matures.

We have made particular use of the Feulgen and the pyronine-methyl green stains in an attempt to follow the invasion of the cell. Sections stained with Feulgen technic (Fig. 2) reveal large amounts of desoxyribonucleic acid (DNA) in the cytoplasm of infected cells, increasing in size and concentration as the inclusion develops. The apparent sequence of events is illustrated by Figures 3, 4 and 5, as shown in selected cells stained by the pyronin-methyl green technic.

In stage 1 of Figure 3 one can see a cell with an enlarged nucleolus and with an increase in diffusely arranged nucleoprotein particularly of the ribonucleic (RNA) type. Stage 2 is much the same picture; minute collections of desoxyribonucleic acid (DNA) are beginning to appear. By stage 3 these collections of desoxyribonucleic acid in the cytoplasm, where this material does not normally,

occur, are clearly apparent. The inclusion body and even the cell are beginning to enlarge due to these growing masses of DNA. In stages 4, 5 and 6 of Figure 4,

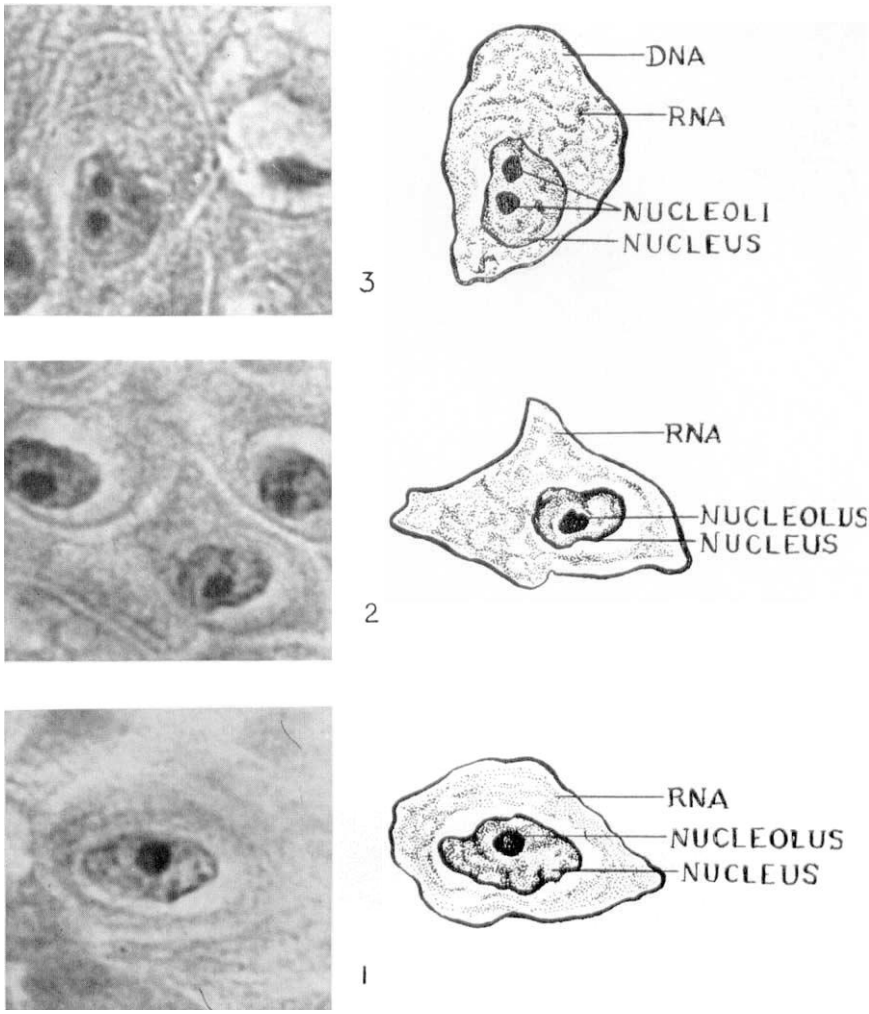

FIG. 3 BIOPSY OF MOLLUSCUM STAINED WITH PYRONIN-METHYL GREEN, X 984

Compare Fig. 3 with Figs. 4 and 5.

Stage 1. An early stage of infection. Active nucleoprotein synthesis has begun as indicated by the enlarged nucleolus, and the increase in ribonucleic acid in the cytoplasm.

Stage 2. The increase in ribonucleic acid in the cytoplasm continues and the cell is beginning to enlarge.

Stage 3. Tiny areas of desoxyribonucleic acid begin to appear in the cytoplasm and begin to push the ribonucleic acid into tiny cords.

the masses of DNA pushing between the RNA force and compress the latter into cords which represent, in the late stages, the trabeculae described by other investigators. The nucleus is forced over to one side of the cell. Finally all that

is left is a cell from which the nucleus has disappeared and in which are seen only masses of DNA separated by trabeculate RNA (Figure 5).

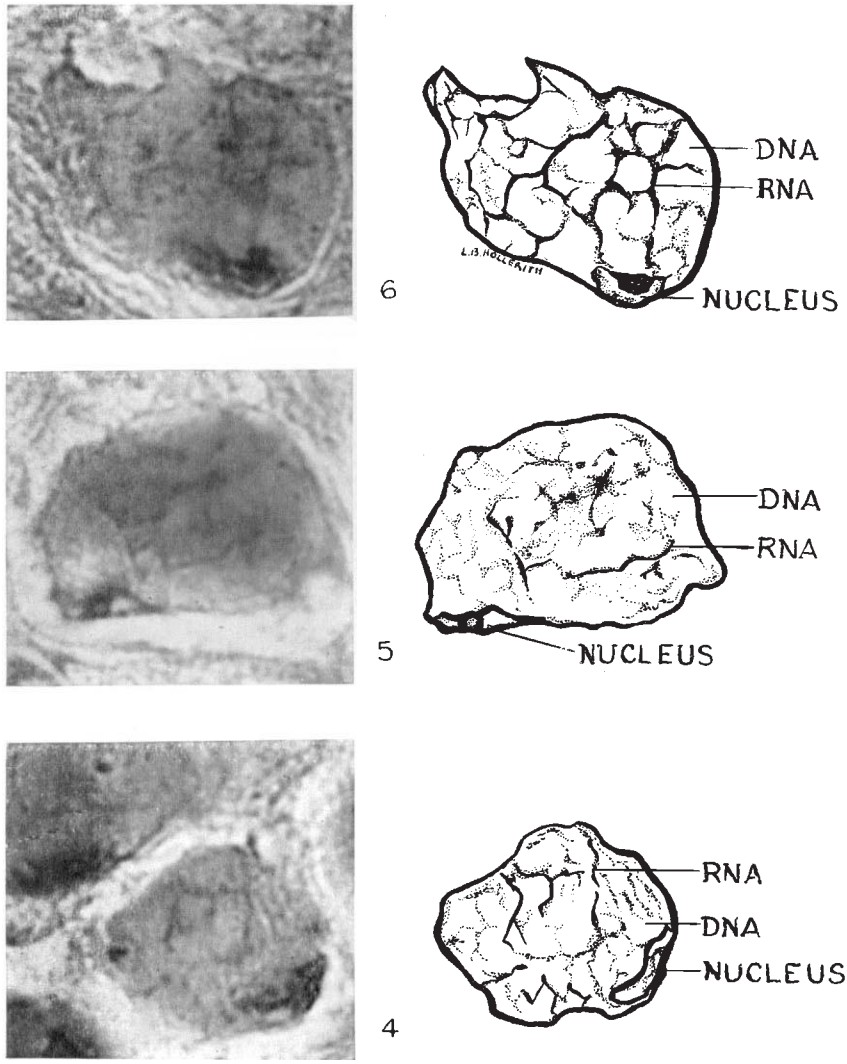

FIG. 4. PYRONIN-METHYL GREEN STAIN × 984

Stage 4. The cytoplasmic islands of desoxyribonucleic acid enlarge further, become more discrete, and force the ribonucleic acid into definite trabeculae. The increase in cytoplasmic nucleoprotein displaces the nucleus to the periphery of the cell.

Stages 5 and 6. Trabeculation of ribonucleic acid continues as the desoxyribonucleic acid increases and enlarges the cell.

These cytochemical studies show that the earliest change in the cytoplasm of the infected cell is one of the synthesis of RNA. The later production is for the most part DNA and it is this increase in DNA which accounts for the marked

increase in size. The large size of the molluscum virus, which is brick shaped and with an average length of 389 mμ, would suggest that it is composed of nucleoprotein of the DNA type. It would indicate therefore that the islands of DNA in the cytoplasm represent actual masses of virus.

The excellent studies of Heyden (10) had already demonstrated the increase of nucleoprotein and the presence of DNA in the cytoplasm. He had, however,

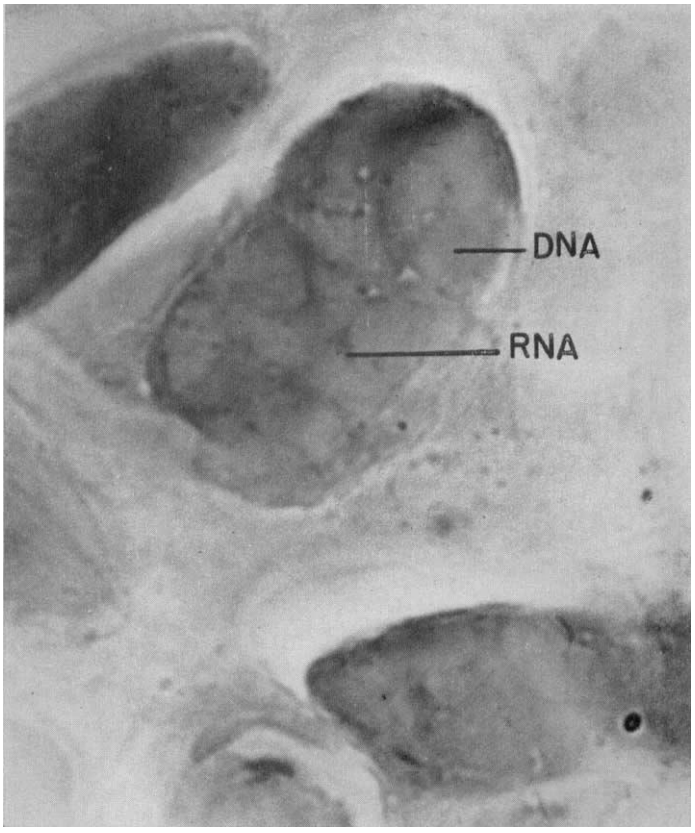

FIG. 5. PYRONIN-METHYL GREEN STAIN × 1200

Fully developed Henderson-Patterson molluscum inclusion body, which consists of an enormous cell which has lost its nucleus and is composed of a large amount of desoxyribonucleic acid separated by trabeculae of ribonucleic acid.

failed to recognize that the earliest material to appear is RNA and that the trabeculae seen in the late stages are physically compressed cords of RNA.

It was clear that studies undertaken with the electron microscope might throw further light on this problem. There are already in the literature several descriptions of the virus of molluscum contagiosum as seen with the electron microscope (12–14). It was obvious from these that the virus elementary bodies were highly plentiful in the lesion; that they were of comparatively large dimensions;

and that morphologically they belonged in the pox group of viruses which group have a characteristic brick shape (15).

In all of these earlier studies, however, examination had been made only of the supernatant suspension of virus after centrifugation procedures of a nature such as to keep only particles or aggregates of a mass approximately that of the molluscum elementary body. It appeared that perhaps more information could be obtained from satisfactory preparations of the material being discarded in the current methods of preparation. The technic described above under materials and methods was therefore adopted.

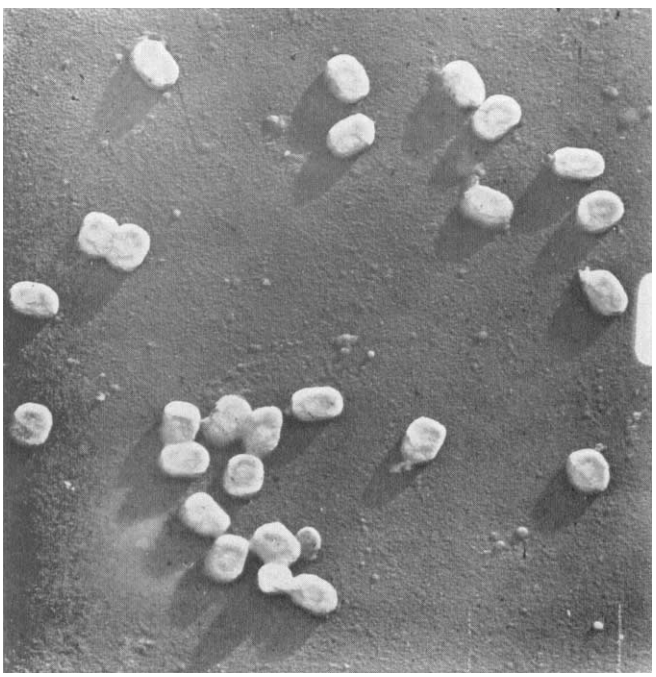

FIG. 6. ELECTRON MICROGRAPH OF MOLLUSCUM CONTAGIOSUM VIRUS SHOWING THE CHARACTERISTIC BRICK SHAPED ELEMENTARY BODIES FOUND IN SUPERNATES OF 2,000 R.P.M. CENTRIFUGED MATERIAL. × 16,667

Before proceeding to discuss the results one should be frank as to certain possible limitations inherent in the technics adopted. The tissue used has been the comedo-like core of the lesion which can readily be expressed. This represents for the most part the fully developed lesion. Only around the edges will the earlier stages be found. The very early changes have probably not been seen and must be sought for by other technics in the spreading edge of the lesions left after the cores have been expressed.

The earlier preparations of 2,000 r.p.m. supernatants of centrifuged material demonstrated for the most part only the characteristic brick shaped elementary bodies (Fig. 6). A uniformity of size and shape is evident, with an apparent differ-

entiation of central structure. Figure 7 is an example of the type of structure that can be found by examination of the 2,000 r.p.m. sediment rather than the supernate (see above under material and technic). This is a mass of material presumably a fragment of an infected cell, in and around which can be seen definite elementary bodies of molluscum contagiosum. They have an average

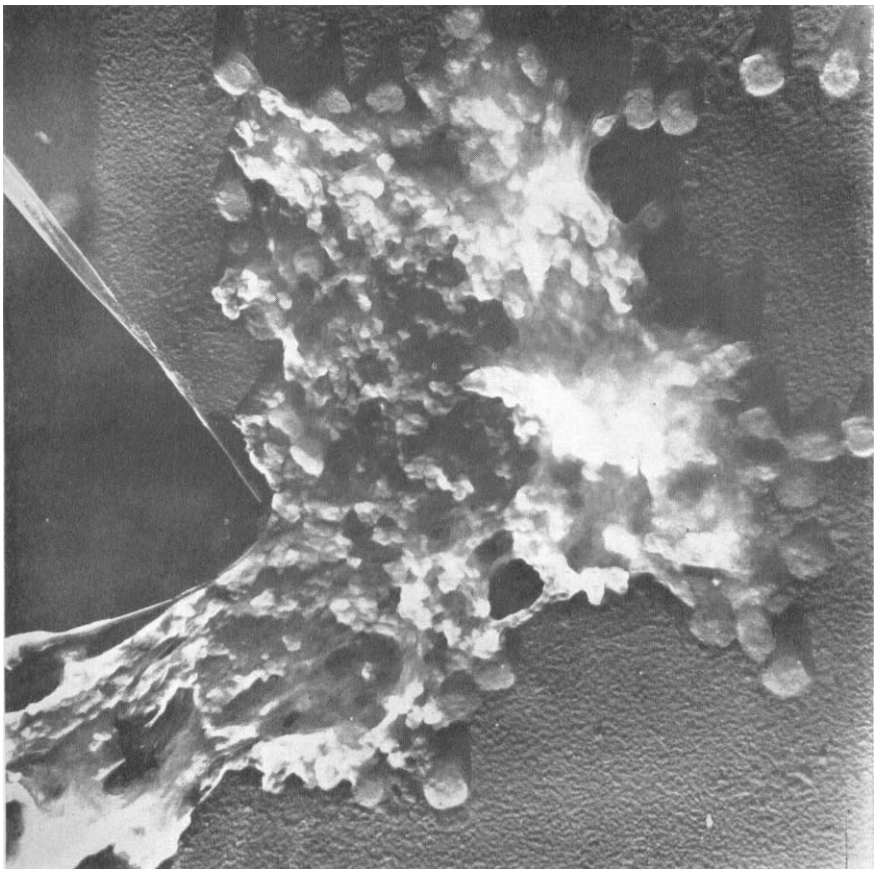

FIG. 7. ELECTRON MICROGRAPH OF SEDIMENT OF 2,000 R.P.M. CENTRIFUGED MOLLUSCUM CONTAGIOSUM MATERIAL

A cell fragment containing definite elementary bodies (averaging 389 x 279 mμ), but also many smaller granules (averaging 100 x 83 mμ). × 15,000.

measurement of 389 x 279 mμ in these shadowed preparations and the readily recognizable structure which can be related to previously known particles. Unlike the elementary bodies shown in Figure 6, however, these present a markedly granular appearance. Most of the granularity is of a fine type but in some bodies larger granules are seen of a somewhat irregular but usually rectangular shape. These correspond in size and shape to the mass of particles making up the central

portion of this "cell fragment." These particles have an average measurement of 100 x 83 mμ in these shadowed preparations and are irregularly arranged although some appear in small aggregations. They as well as some of the elementary bodies appear to be embedded in an undifferentiated material with a physical appearance suggesting a stringy, "sticky," nature.

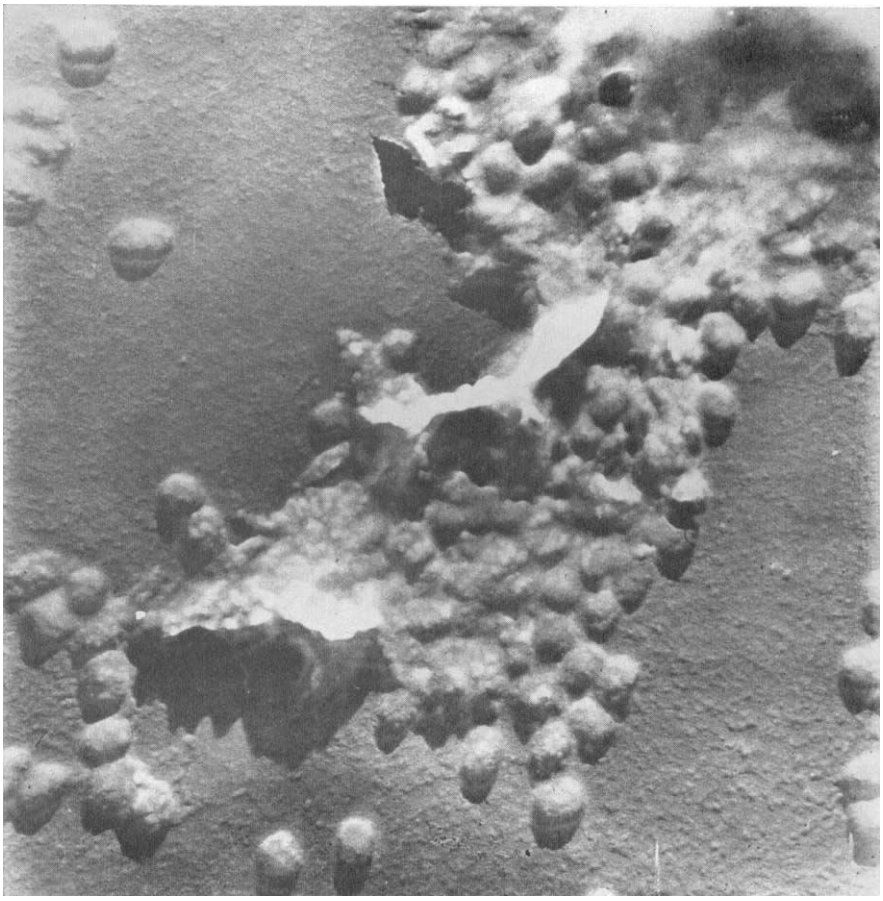

FIG. 8. ELECTRON MICROGRAPH OF SEDIMENT OF 2,000 R.P.M. CENTRIFUGED MOLLUSCUM CONTAGIOSUM

The smaller "sub-virus" granules and the granularity of the formed virus particles is well shown. × 15,000.

Figure 8 is another characteristic fragment. Unlike the fragment in Figure 7 there has been no physical stress (in Figure 7 perhaps the tearing of the supporting collodion membrane was responsible) to tear the fragment apart. We lack in Figure 8, therefore, the capacity to look inside the fragment which we appear to have in Figure 7. Again elementary bodies can be recognized free around the fragment or embedded in it. The granularity of these elementary bodies is even

more apparent in Figure 8 than in Figure 7. Toward the center of the fragment the elementary bodies are less distinct, in some cases appearing as little more than aggregates of granules embedded in little differentiated material and with but vague outlines.

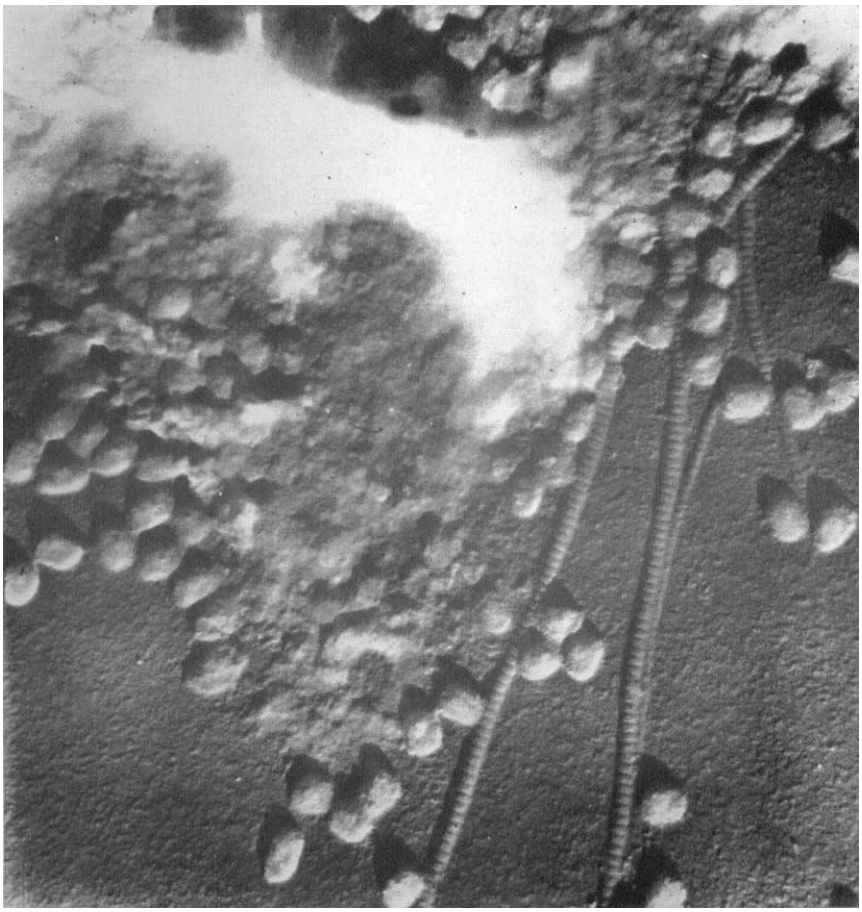

FIG. 9. ELECTRON MICROGRAPH OF SEDIMENT OF 2,000 R.P.M. CENTRIFUGED MOLLUSCUM CONTAGIOSUM

The smaller granules are most numerous toward the center of the fragment with varying sized aggregates of the granules, some approximately the same size as the fully formed virus, near the periphery. Note the fibrils with clear and consistent periodic structure. × 15,000.

Figure 9 shows an appearance similar to that of Figure 8. The elementary bodies are distinct in outline and characteristic in size and shape around the periphery; in the more central part of the fragment there is little distinction between the elementary bodies of somewhat irregular shape and vague outline and aggregations of granules, which aggregations are often approximately the

same size as the fully formed elementary body. In this figure one can also see the distinction between fully formed elementary bodies which are relatively homogeneous, at least except for their central nucleus, and the "less mature" forms at the margin of the fragment or still embedded in it with granular structure and marked irregularity.

Also present in Figure 9 and worthy of brief comment are fibrils with clear and consistent periodic structure. Some of these are seen on the periphery of the fragment where they differ considerably in diameter. Others, and still thinner ones may be made out embedded in the fragment. These fibrils correspond in every particular to collagen fibrils although it is a matter of some surprise to find collagen in these preparations of presumably pure squamous epithelium. On the other hand, Lepine and his coworkers state that the periodicity of collagen, nerve fiber and ribonucleoprotein is always the same within limits of statistical errors of determination (11). The fibrils seen in molluscum, therefore, may represent fragments of the ribonucleoprotein trabeculae demonstrated histochemically.

In summary of the appearances under the electron microscope (and it may be said that the 3 micrographs are selected from over a hundred taken) there appear to be three main structures—the elementary bodies, the rectangular granules and the "sticky," relatively undifferentiated, background substance or matrix. In general the central portion of all tissue fragments examined contains for the most part the rectangular granules embedded in the matrix. Free at the periphery are elementary bodies similar to or identical with those found in the usual preparations of supernate. In the zone intermediate between these two the picture is less clear. There is, however, a suggestion that aggregations of the rectangular granules are the prime elements around which the elementary bodies form. There is little within the tissue fragments to indicate binary fission of elementary bodies.

SUMMARY AND CONCLUSIONS

1. Chemical and physical changes which occur with infection of human epithelial cells with the virus of the molluscum contagiosum have been studied with histochemical and electron microscope technics.

2. The earliest stages of infection are manifested by an increase in ribonucleic acid (RNA) synthesis with an increase in cytoplasmic ribonucleo-protein and enlargement of the nucleolus.

3. Subsequently islands of desoxyribonucleic acid (DNA) protein appear in the cytoplasm and increase to enormous proportions, displacing all cell structures and producing the characteristic inclusion body. It is suggested that this consists primarily of a mass of virus particles.

4. The cytoplasmic trabeculae which appear are cords of RNA apparently physically compressed by the DNA.

5. The fully formed brick shaped virus particles (elementary bodies) with an average measurement when shadowed of 389 x 279 mμ are extremely plentiful in the molluscum inclusion bodies.

6. The fully formed virus particles are relatively homogeneous but the 'less mature" forms exhibit a marked granular structure.

7. The virus particles seem to be formed by an aggregation of smaller particles with an average measurement when shadowed of 100 x 83 mμ. These "sub-virus" bodies give the virus particle its granular appearance. The technic of demonstrating these units is described.

8. The "sub-virus" granules in all stages of aggregation and the fully formed virus particles seem to be embedded in a "stringy," "sticky," material.

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